BacPAK™ Baculovirus Expression System

- *High-level expression—1 to 500 mg of protein per liter of culture*
- *Post-translational processing similar to mammalian cells—folding, disulfide bond formation, glycosylation, acylation, phosphorylation, and signal peptide cleavage*
- *Quick and easy to use—insect cells grow rapidly in monolayers or suspension cultures and do not require CO_2 for growth*
- *Safe—baculoviruses only replicate in insect cells; they do not infect humans, animals, or plants*

Figure 12.3. Relative efficiency of producing recombinant viruses using wild-type or BacPAK6 viral DNAs. Sf21 cells were cotransfected with pBacPAK8-GUS (a transfer vector containing the *E. coli* β-glucuronidase [GUS] gene) and either circular wild-type AcMNPV DNA (Panel A) or *Bsu*36 I-digested BacPAK6 DNA (Panel B). Viruses produced after 48–72 hr were harvested and plated to obtain individual plaques. In the presence of X-Gluc and neutral red, recombinant plaques turn blue, nonrecombinant plaques appear white, and uninfected cells stain red.

The baculovirus expression system can be used to produce large amounts of target protein in insect host cells. It offers a number of advantages over prokaryotic, yeast, and mammalian expression systems (Table 12.2), and is rapidly becoming the system of choice for expressing eukaryotic proteins. More than 300 different proteins have been expressed using baculovirus vectors (3), and in most cases, the protein produced is similar in structure, biological activity, and immunological reactivity to the naturally occurring protein.

Baculovirus expression systems use very strong viral promoters that are activated in the final phase of baculovirus infection to drive expression of cloned genes in insect cells. Construction of recombinant baculovirus expression vectors has been greatly facilitated at CLONTECH by the development of specially adapted viruses (1–2) by Paul Kitts, Ph.D. The BacPAK Baculovirus Expression System features the most advanced virus and most versatile transfer vectors to allow easy introduction of the target gene. In addition, pBacPAK-His Transfer Vectors, which generate a 6xHis-tagged protein, are available separately and allow easy purification of the expressed protein using CLONTECH's TALON™ resin or other IMAC resin. (See pages 193–194 for information about TALON.)

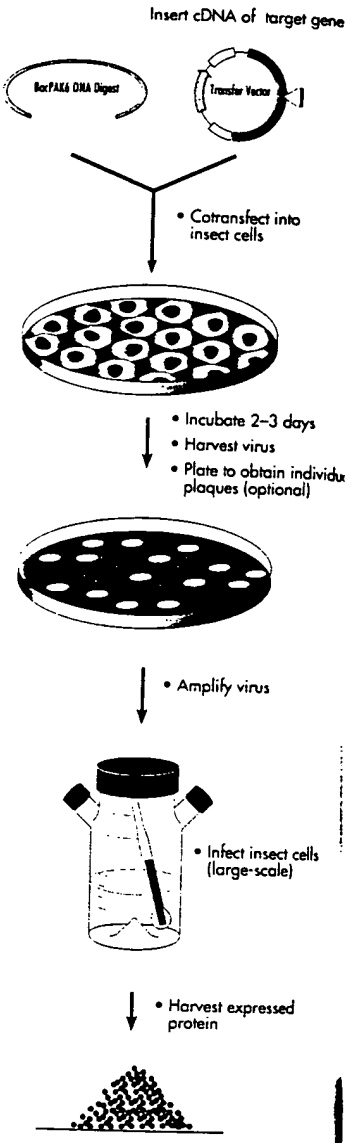

Figure 12.4. Flow chart of the BacPAK Baculovirus System.

Comparison of Protein Production in Various Expression Systems

EXPRESSION SYSTEM	*E. coli*	YEAST CELLS	MAMMALIAN CELLS	INSECT CELLS
Proteolytic cleavage	+/–	+/–	+	+
Glycosylation	–	+	+	+
Secretion	+/–	+	+	+
Folding	+/–	+/–	+	+
Phosphorylation	–	+	+	+
Acylation	–	+	+	+
Amidation	–	–	+	+
Percent Yield (based on dry weight)	1–5%	1%	<1%	30%

Table 12.2. Post-translational processing and yields of proteins produced in various expression systems. Adapted from Vlak & Keus (1990).

REFERENCES

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*This book is available from CLONTECH: Cat. #V2065-1.